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Molecular lesions associated with *white* gene mutations induced by I-R hybrid dysgenesis in *Drosophila melanogaster*

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We have identified molecular lesions associated with six mutations, w^{IR2} and w^{IR4-8} , of the *white* gene of *Drosophila melanogaster*. These mutations arose in flies subject to I-R hybrid dysgenesis. Four of the mutations give rise to coloured eyes and are associated with insertions of 5.4-kb elements indistinguishable from the I factor controlling I-R dysgenesis. The insertion associated with w^{IR4} is at a site which, within the resolution of these experiments, is identical to that of two previously studied I factors. This appears to be a hot-spot for I factor insertion. We have compared the sites of these insertions with sequences complementary to *white* gene mRNA identified by Pirrotta and Bröckl. The hot-spot is in the fourth intron. The insertion carried by w^{IR5} is either within, or just beyond, the last exon. The insertion carried by w^{IR6} is near the junction of the first exon and first intron. The w^{IR2} mutation is a derivative of w^1 . It contains an insertion of I factor DNA within, or immediately adjacent to, the F-like element associated with w^1 , and results in restoration of some eye colour. This insertion is just upstream of the start of the *white* mRNA. Mutations w^{IR7} and w^{IR8} are deletions removing mRNA coding sequences. Both determine a bleached white phenotype.

Key words: *Drosophila melanogaster*/hybrid dysgenesis/transposable elements/*white* gene

Introduction

Hybrid dysgenesis (see reviews by Bregliano and Kidwell, 1983; Kidwell, 1983; Engels, 1983) is the name given to the appearance of a set of unusual characteristics in the progeny of crosses between certain strains of *Drosophila melanogaster* (Picard and L'Héritier, 1971; Kidwell, 1975; Kidwell *et al.*, 1977). These characteristics include lowered fertility, recombination in males and increased frequencies of mutation and chromosome aberrations. Two independent systems of hybrid dysgenesis, P-M and I-R, are known (Kidwell, 1979). Strains of *D. melanogaster* may be classified into one or other of two types with respect to each system. In the P-M system the effect is seen in male and female progeny of crosses between M strain females and P strain males. In the I-R system the effect is seen only in the female progeny of crosses between reactive (R) strain females and inducer (I) strain males. These dysgenic females are known as 'SF' females. The progenies of all other crosses appear normal.

The characteristics of P strains and inducer strains are controlled by transposable genetic determinants known as P fac-

tors and I factors, respectively (Picard, 1976; Bingham *et al.*, 1982). Many P-M induced mutations are unstable in individuals subject to P-M dysgenesis (Engels, 1979; Rubin *et al.*, 1982) and this led to the suggestion that mutations induced by P-M dysgenesis are due to insertion of P factor DNA into the genes in question (Green, 1977; Golubovsky *et al.*, 1977; Simmons and Lim, 1980). This has been tested by Rubin *et al.*, 1982). They compared DNA of six P-M induced *white* gene mutations with that of the wild-type allele. Each mutation had foreign DNA inserted into the *white* gene. Two insertions were of members of the *copia* family of transposable elements (Finnegan *et al.*, 1978). The other four insertions varied in length from 0.5 kb to 1.4 kb, but were related in sequence. Rubin *et al.* (1982) argued that these insertions were too short to be functional P factors, which probably code for at least a transposase and a regulatory molecule, but that they might be deleted derivatives of P factors. O'Hare and Rubin (1983) have confirmed this. Using one of these putative P elements as a probe to screen a library of recombinant phages containing DNA from a P strain they recovered clones containing a conserved 2.9-kb sequence which has subsequently been shown to have at least some of the properties of a P factor (Spradling and Rubin, 1982).

We have started to investigate the molecular basis of I-R hybrid dysgenesis by examining molecular lesions associated with mutations of the *white* gene produced in SF females. Two mutations, w^{IR1} and w^{IR3} , are associated with insertions of indistinguishable 5.4-kb elements at apparently identical sites within the *white* gene (Bucheton *et al.*, 1984). We believe that these insertions are copies of the I factor, which controls I-R dysgenesis, since both w^{IR1} and w^{IR3} are closely linked to I factor activity (Pélisson, 1981; Bucheton *et al.*, 1984). Here we describe the genetical and molecular properties of a further six *white* gene mutations induced in SF females. Three of these mutations, w^{IR4} , w^{IR5} and w^{IR6} , determine a coloured eye phenotype and are associated with insertions of 5.4-kb elements which are very similar, if not identical, to the I factor. Two of the remaining mutations, w^{IR7} and w^{IR8} , determine a bleached white phenotype and are associated with deletions of DNA from the *white* region. The last mutation, w^{IR2} , contains an insertion of I factor DNA into the *white* region of a chromosome carrying the w^1 allele. This results in a partial restoration of eye colour. We discuss these results in terms of the properties of the I factor and of the *white* gene.

Results

The mutation w^{IR2} , like w^{IR1} , arose in SF females produced by crossing females of the reactive strain *seF₈* with males of the inducer strain w^1 *ct f* (Picard *et al.*, 1978; Pélisson, 1981). Its properties have been described briefly by Pélisson (1981) who referred to it as 1a11. Mutations w^{IR4-8} were found amongst 60 000 male progeny of SF females produced by crossing females of the reactive strain XCha with males of the inducer strain XOré I. They are the results of independent mutation events. Mutations w^{IR2} and w^{IR4-6} determine col-

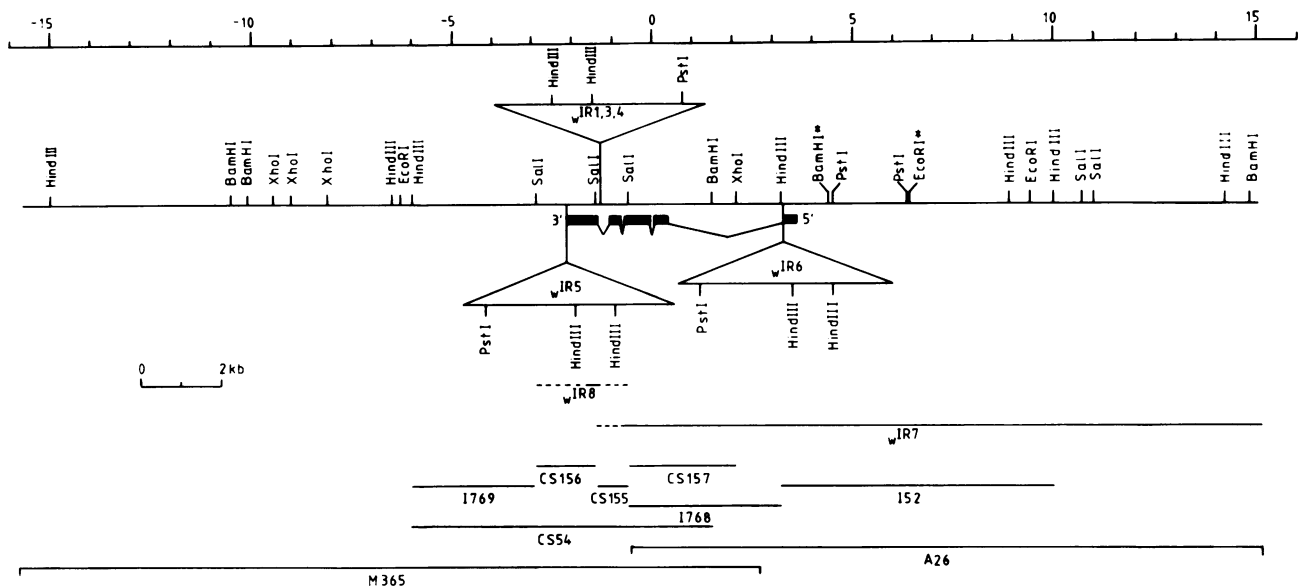


Fig. 1. Restriction map of the *white* region in chromosomes carrying w^{IR} mutations. This map shows the positions of restriction sites on the parental w^+ chromosomes on which w^{IR} mutations were isolated. The positions of the insertions in w^{IR4} , w^{IR5} and w^{IR6} are shown offset from the map. The positions of the deletions associated with the w^{IR7} and w^{IR8} mutations are shown below the map. The positions of the deletions associated with the w^{IR7} and w^{IR8} mutations are shown below the map. The dashed regions indicate uncertainties regarding the end points of the deletions. Restriction sites marked * are not present on the chromosome carrying the w^{IR4} and w^{IR5} mutations, but are present on the chromosome carrying w^{IR6} . The *HindIII* site at coordinate 8.9 is not present in chromosomes carrying the w^1 or w^{IR2} mutations. The positions of fragments which have been cloned from the *white* region and used as probes in these experiments are also indicated below the map. The scale above the map is in kilobases. The zero position is the site of the *copia* element associated with the *white-apricot* mutation (Levis *et al.*, 1982). Positive coordinates are towards the centromere. The position of the major w^+ transcript as determined by Pirrotta and Brocchi (1984) is shown under the restriction map. The boxed regions indicate exons while the lines joining them indicate introns. Transcription is from right to left.

oured eye phenotypes and all show dosage compensation.

We first determined the approximate locations of molecular lesions associated with these mutations by restriction digestion and Southern (1975) transfer experiments. Initially, recombinant lambda phages A26 and M365 (Figure 1) were used to probe digests of mutant and wild-type DNAs. These phages together cover ~30 kb of the *white* locus. Subclones of appropriate restriction fragments allowed a more detailed analysis.

Properties of the mutation w^{IR4}

The mutation w^{IR4} determines a red-brown eye colour which is lighter at 25°C than at 20°C. This phenotype is identical to that of w^{IR1} and w^{IR3} (Pélisson, 1981, and unpublished). The only detectable difference between wild-type and w^{IR4} DNAs in the region of the *white* gene is within the 0.86-kb *SalI* fragment lying between coordinates -1.4 and -0.6 in Figure 1. This is the location of the 5.4-kb insertion associated with the w^{IR1} and w^{IR3} mutations (Bucheton *et al.*, 1984). Figure 2, tracks a–d, shows the result of a Southern transfer experiment in which wild-type, w^{IR1} , w^{IR3} and w^{IR4} DNAs were digested with *SalI* and then probed with 32 P-labelled pCS155, a plasmid containing this 0.86-kb fragment. The 0.86-kb *SalI* fragment of wild-type DNA is replaced by a 6.2-kb fragment in w^{IR4} DNA which co-migrates with the corresponding fragments of w^{IR1} and w^{IR3} . This indicates that these mutations are all associated with 5.4-kb insertions in this region.

The 5.4-kb elements present in w^{IR1} and w^{IR3} are apparently identical copies of the I factor. These have been cloned and characterised by Bucheton *et al.* (1984). They are not cut by *BamHI*, *EcoRI*, *SalI* or *XhoI* and the same is true of the element present in w^{IR4} (data not shown). The copies of the I factor in w^{IR1} and w^{IR3} have two *HindIII* sites 1 kb apart, and in order to compare the element in w^{IR4} with these I factors

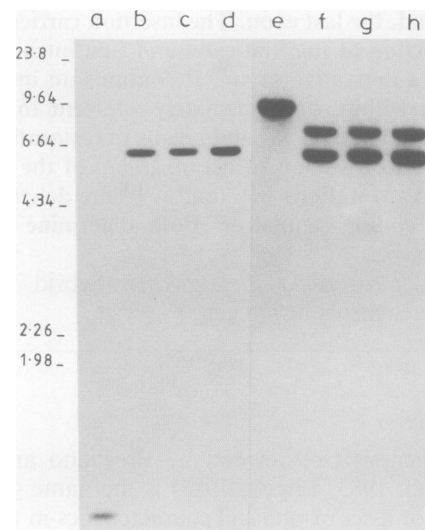


Fig. 2. Southern transfer experiment comparing the w^{IR1} , w^{IR3} and w^{IR4} mutations. Genomic DNAs of *seF₈*, w^{IR1} , w^{IR3} and w^{IR4} strains were digested with *SalI*, tracks a–d, or *HindIII*, tracks e–h. The DNAs were then run on a 1% agarose gel and transferred to nitrocellulose. The DNAs in tracks a–d were hybridised with 32 P-labelled pCS155, while DNAs in tracks e–h were hybridised with 32 P-labelled pCS54. The strain *seF₈* has the same restriction map in the *white* region as does the parental strain XChA. a and e, *seF₈*; b and f, w^{IR1} ; c and g, w^{IR3} ; d and h, w^{IR4} .

we digested wild-type, w^{IR1} , w^{IR3} and w^{IR4} DNAs with *HindIII*. The DNAs were then hybridised, in a Southern transfer experiment, with pCS54, a plasmid carrying the *BamHI*-*HindIII* fragment lying between coordinates -6 and 1.5 in Figure 1. The 9.2-kb *HindIII* fragment of wild-type DNA which hybridises to this probe is replaced, in the three mutant DNAs, by fragments of 7.4 kb and 6.2 kb (Figure 2,

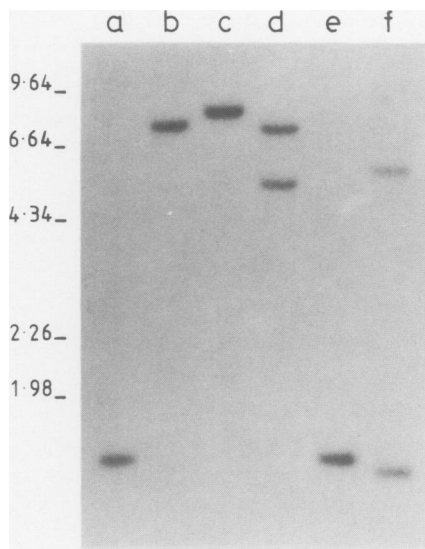


Fig. 3. Southern transfer experiment showing the position of the insertion associated with the w^{IR5} mutation. Genomic DNAs of the seF_8 and w^{IR5} strains were digested with the enzymes indicated below. The DNAs were then run on a 1% agarose gel, transferred to nitrocellulose filter and hybridised with ^{32}P -labelled pCS156. **a**, seF_8 digested with *SalI*; **b**, w^{IR5} digested with *SalI*; **c**, seF_8 digested with *Bam*HI and *Hind*III; **d**, w^{IR5} digested with *Bam*HI and *Hind*III; **e**, seF_8 digested with *SalI* and *Pst*I; **f**, w^{IR5} digested with *SalI* and *Pst*I.

tracks e–h). This suggests that w^{IR4} contains a copy of the same 5.4-kb element as is present in w^{IR1} and w^{IR3} , and that it is inserted at the same site and in the same orientation. We refer to this orientation of the I factor with respect to the *white* locus as being orientation 1, and to the opposite as orientation 2.

Properties of the mutation w^{IR5}

The mutation w^{IR5} determines a brown eye colour which is similar to that of w^{IR1} at 20°C, but it is not temperature sensitive. The only detectable difference between the *white* regions of wild-type and w^{IR5} DNAs is in the 1.5-kb *SalI* fragment between coordinates –2.9 and –1.4. Figure 3, tracks a and b, shows the result of hybridising *SalI* digests of wild-type and w^{IR5} DNAs with pCS156, a plasmid containing this fragment. The 1.5-kb *SalI* fragment of wild-type DNA is replaced by a 6.9-kb fragment in w^{IR5} . This indicates that this mutation is also associated with insertion of a 5.4-kb element.

The insertion in w^{IR5} is not cut by *Bam*HI, *Eco*RI, *Sal*I or *Xho*I (data not shown) but is cut by *Hind*III. Figure 3, tracks c and d, shows the result of digesting wild-type and w^{IR5} DNAs with both *Hind*III and *Bam*HI and then hybridising them with pCS156 in a Southern transfer experiment. The 7.8-kb fragment of wild-type DNA which hybridises to this probe is replaced in w^{IR5} by fragments of 7 kb and 5.2 kb. This indicates that, like the I factor in w^{IR1} , the 5.4-kb element in w^{IR5} contains at least two *Hind*III sites ~1 kb apart. This suggests that the element present in w^{IR5} is another copy of the I factor.

To orient and position this element we hybridised a *Hind*III–*Bam*HI digest of w^{IR5} DNA separately with plasmid pI769, which contains the *Hind*III–*Sal*I fragment between coordinates –6 and –2.9, and pCS157, which contains the *Sal*I–*Xho*I fragment between coordinates –0.6 and 2.1 (Figure 1). The 7-kb *Hind*III fragment hybridised to pI769 and the 5.2-kb fragment to pCS157 (data not shown). The

7.0-kb fragment is therefore to the left and the 5.2-kb fragment to the right. If the element in w^{IR5} has the same restriction map as the I factor in w^{IR1} then it must be inserted at about coordinate –2 and in orientation 2. This would place a *Pst*I site ~1.6 kb to the right of the *Sal*I site at coordinate –2.9, and 5.3 kb to the left of the *Sal*I site at coordinate –1.4 (Figure 2). To position the insert more precisely we digested wild-type and w^{IR5} DNAs with both *Sal*I and *Pst*I and hybridised them with pCS156 (see Figure 3, tracks e and f). The 1.5-kb *Sal*I fragment of wild-type DNA which hybridises to this probe is replaced by 1.4-kb and 5.5-kb fragments in w^{IR5} DNA, which places the w^{IR5} insert at about coordinate –2.2.

We have cloned the *Hind*III fragments containing the left-hand and right-hand ends of the w^{IR5} insert, using the lambda vector NM1149 (Murray, 1983). The recombinant phages obtained, λ I453 and λ I454, contain the left-hand and right-hand ends of the insert, respectively. Digestion of λ I453 with both *Hind*III and *Sal*I yields a fragment of 3.7 kb which includes the left-hand end of the insert, while digestion of λ I454 gives a 2.2-kb fragment including the right-hand end. To compare the insert present in w^{IR5} directly with the I factor in w^{IR1} , we subcloned these *Sal*I–*Hind*III fragments in pUC8 (Vieira and Messing, 1982) and heteroduplexed them with the corresponding subclones from w^{IR1} . The results of this experiment indicate that the w^{IR5} insertion is homologous to I factor DNA for 1.4 ± 0.14 kb to the left of the first internal *Hind*III site of the I factor, as shown in Figure 1, and for 2.8 ± 0.09 kb to the right of the second internal *Hind*III site. No region of mismatch could be detected. These results are consistent with the w^{IR5} insertion being an I factor.

Properties of the mutation w^{IR6}

The mutation w^{IR6} determines a red-brown eye colour which is similar to that of w^{IR1} at 20°C, but which is not temperature sensitive. The only detectable difference between w^{IR6} and wild-type DNAs is in the 11.3-kb *Sal*I fragment between coordinates –0.6 and 10.7 (Figure 1). This is replaced in w^{IR6} DNA by a fragment of ~17 kb (data not shown). This is consistent with there being a 5.4-kb I factor inserted within this fragment. We have confirmed that there are I factor sequences associated with the w^{IR6} mutation by *in situ* hybridisation experiments (data not shown).

There are differences between the restriction maps of the inducer and reactive parents of w^{IR6} in this region. The *Bam*HI site at coordinate 4.4 and the *Eco*RI site at coordinate 6.4 are both present in DNA of the X^{Ore} I, but not the X^{Cha} parent. The DNA from w^{IR6} contains both sites indicating that this mutation occurred on an X chromosome from X^{Ore} I. We have further located the w^{IR6} insertion by hybridising *Hind*III digests of w^{IR6} and X^{Ore} I DNAs with pI52, a plasmid containing the 6.8-kb *Hind*III fragment lying between coordinates 3.2 and 10. This was cloned from a strain lacking the *Hind*III site at coordinate 8.9. This probe hybridised to 5.7-kb and 1.1-kb fragments of X^{Ore} I DNA, and 7.1-kb and 1.1-kb fragments of w^{IR6} DNA (Figure 4, tracks a and b). Therefore the insertion must be within the 5.7-kb fragment, and must contain at least one *Hind*III site. The fact that only one new fragment of w^{IR6} DNA hybridises to this probe probably indicates that the insert is very close to one end of the *Hind*III fragment. These results suggest that an I factor is present just to the right of the *Hind*III site at coordinate 3.2 and in orientation 2, or just to the left of the *Hind*III site at coordinate 8.9 and in orientation 1.

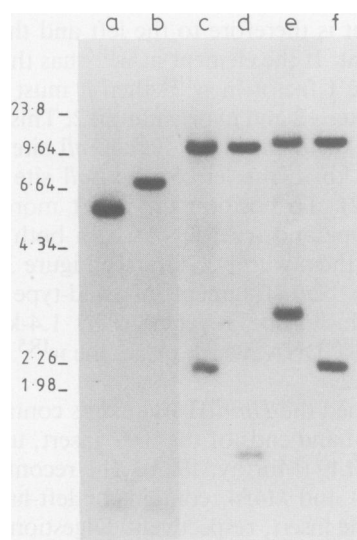


Fig. 4. Southern transfer experiment showing the position of the insertion associated with the w^{IR6} mutation. Genomic DNAs of the XOre I and w^{IR6} strains were digested with the enzymes indicated below. The DNAs were then run on a 1% agarose gel and hybridised with 32 P-labelled p152, tracks a and b, or p1768, tracks c–f. a, XOre I digested with *Hind*III; b, w^{IR6} digested with *Hind*III; c, XOre I digested with *Pst*I and *Xho*I; d, w^{IR6} digested with *Pst*I and *Xho*I; e, XOre I digested with *Bam*HI and *Pst*I; f, w^{IR6} digested with *Bam*HI and *Pst*I.

We have determined the position and orientation of this insert by hybridising *Xho*I–*Pst*I and *Bam*HI–*Pst*I digests of XOre I and w^{IR6} DNAs with p1768, a plasmid carrying the *Sal*I–*Hind*III fragment lying between coordinates –0.6 and 3.2 (Figure 1). The results are shown in Figure 4, tracks c–f. The 2.3-kb *Xho*I–*Pst*I (coordinates 2.1–4.4), and the 2.9-kb *Bam*HI (coordinates 1.5–4.4) fragments of XOre I DNA are replaced by 1.7-kb and 2.3-kb fragments, respectively, in w^{IR6} DNA. These results are consistent with an I factor being present at about coordinate 3.2 and in orientation 2 (Figure 1).

Mutations w^{IR7} and w^{IR8} are deletions

Mutations w^{IR7} and w^{IR8} determine a bleached white phenotype. Chromosomes carrying the w^{IR7} mutation do not complement mutations at the *roughest* gene which lies proximal to *white*. This suggests that w^{IR7} is associated with a long deletion. We have confirmed this at the molecular level.

Figure 5, tracks a and b, shows the result of hybridising a *Sal*I digest of wild-type and w^{IR7} DNAs with pCS54. Both DNAs contain the 1.5-kb *Sal*I fragment between coordinates –2.9 and –1.4, and the large fragment of ~20 kb to the left of it. Only wild-type DNA contains the 0.86-kb fragment between coordinates –1.4 and –0.6 and the 11.3-kb fragment between coordinates –0.6 and 10.7, indicating that w^{IR7} has lost DNA in this region. A faint fragment of ~12 kb can be seen in w^{IR7} DNA. This is presumably a fusion fragment generated by the deletion. The fact that it is so faint suggests that the left-hand breakpoint of the deletion is close to the *Sal*I site at coordinate –1.4. We cannot detect any homology between w^{IR7} DNA and λ A26 so the deletion must extend proximally beyond the limits of this phage, probably at least as far as the *roughest* gene.

The only difference we have detected between wild-type and w^{IR8} DNAs is in the region between coordinates –2.9 and –0.6. Neither the 1.5-kb nor the 0.86-kb *Sal*I fragments are present in w^{IR8} indicating that a deletion has removed

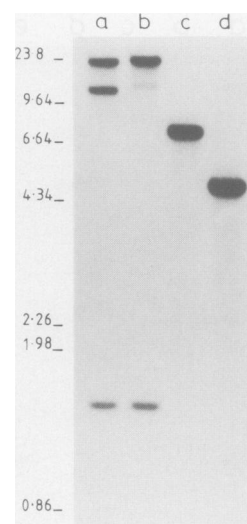


Fig. 5. Southern transfer experiment showing the deletions associated with the w^{IR7} and w^{IR8} mutations. Genomic DNAs of strains *seF*₈, w^{IR7} and w^{IR8} were digested with the enzymes indicated below. The DNAs were then run on a 1% agarose gel, transferred to nitrocellulose filter and hybridised with 32 P-labelled pCS54. a, *seF*₈ digested with *Sal*I; b, w^{IR7} digested with *Sal*I; c, *seF*₈ digested with *Bam*HI and *Hind*III; d, w^{IR8} digested with *Bam*HI and *Hind*III.

DNA in this region. This includes the *Sal*I site at coordinate –1.4 (data not shown). We have measured the extent of this deletion by hybridising *Bam*HI–*Hind*III digests of wild-type and w^{IR8} DNAs with pCS54. The 7.8-kb fragment of wild-type DNA is replaced by a fragment of ~4.9 kb in w^{IR8} DNA (Figure 5, tracks c and d). This confirms the presence of a deletion in this region and indicates that it is ~2.5 kb long. The *Sal*I sites at coordinates –2.9 and –0.6 are still present since digestion of w^{IR8} DNA with both *Hind*III and *Sal*I yields fragments which co-migrate with the *Hind*III–*Sal*I fragments of wild-type DNA between coordinates –6.0 and –2.9, and between –0.6 and 3.2, and which hybridise to pCS54 (data not shown).

Properties of the mutation w^{IR2}

The mutation w^{IR2} arose in an SF female resulting from a cross between *seF*₈ females and w^1 *ct f* males. It determines a brown eye phenotype which is not temperature sensitive. The chromosome initially carrying w^{IR2} also carried the *ct* and *f* markers, suggesting that the w^{IR2} mutation occurred in an X chromosome from the inducer parent and that it is a partial revertant of w^1 .

The w^1 mutation is associated with insertion of a 5.7-kb element between coordinates 3.1 and 4.4 in Figure 1 (Zachar and Bingham, 1982). This insertion is related to transposable elements known as 'F' elements (O'Hare and Rubin, 1983; Di Nocera *et al.*, 1983). We have investigated the structure of the *white* locus in chromosomes carrying w^{IR2} by hybridising digests of w^1 *ct f* and w^{IR2} DNAs with 32 P-labelled λ A26 and λ M365. The results indicated that w^{IR2} contains all or part of the w^1 insertion together with additional DNA. The second insertion is either just within, or just to the right of, the w^1 insertion. We have cloned, from w^{IR2} DNA, the *Bam*HI fragment lying between coordinates 1.5 and 4.4 using the lambda vector EMBL4 (Frischauf *et al.*, 1983). This yielded the recombinant phage λ I531. Figure 6 is a restriction map of the *Bam*HI fragment carried by this phage. This fragment includes both the w^1 and w^{IR2} insertions. It contains I factor se-

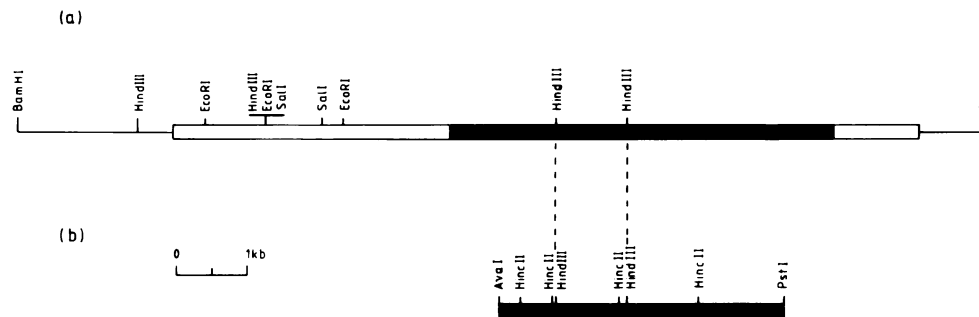


Fig. 6. Restriction map of the *Bam*HI fragment containing the w^1 and w^{IR2} insertions. The upper part of the figure, (a), is a restriction map of the *Bam*HI fragment cloned in λ I531. The *Bam*HI sites at the ends of this fragment correspond to those at coordinates 1.5 and 4.4. in Figure 1. The lower part of the figure, (b), is a more detailed map of the region of λ I531 containing I factor sequences. Restriction fragments in this region co-migrate with, and hybridise to, the corresponding fragments of a cloned I factor. The broad line in (a) indicates DNA inserted at the *white* locus in association with the w^{IR2} mutation. It includes both F element and I factor DNA. Its position and length have been deduced from data concerning the w^1 and w^e mutations given by Zachar and Bingham (1982) and Pirrotta and Bröckl (1984). The map of the region corresponding to the w^1 insertion is indicated by the open broad line. The solid broad line indicates the probable extent of I factor sequences. The length and position of this region has been deduced from the length of I factor sequences in λ I531 as determined by heteroduplex experiments (see text) and comparison of the restriction map shown in (b) with that of a cloned I factor. The relative order of restriction sites enclosed in brackets has not been determined. Sites within a cluster are no more than ~ 100 bp apart.

quences since it hybridises to I factor probes and has a region with a restriction map which corresponds to that of the I factor from w^{IR1} (Bucheton *et al.*, 1984). This region is indicated in Figure 6. To measure the total length of I factor sequences present in w^{IR2} , we heteroduplexed λ I531 with pI407, a plasmid containing the complete I factor from w^{IR3} (Bucheton *et al.*, 1984). An uninterrupted double-stranded region of 5.4 ± 0.24 kb long was formed. These results are consistent with there being a complete I factor within the w^1 insertion. The restriction map of the region of λ I531 corresponding to the w^1 insertion is not identical to that published by Zachar and Bingham (1982) but, so far as we can tell from Southern transfer experiments, it is the same as the equivalent regions of genomic w^{IR2} and parental w^1 *ct f* DNAs.

Discussion

All of the *white* gene mutations described here are associated with DNA rearrangements. We have no formal proof that these rearrangements are responsible for the mutant phenotypes associated with the mutations but this seems very likely. We have compared the molecular lesions associated with the w^{IR} mutations with information concerning transcription of the *white* gene. Pirrotta *et al.* (1983), O'Hare *et al.* (1983) and Pirrotta and Bröckl (1984) have mapped the regions of the *white* locus which are complementary to mRNA. Transcription is from right to left in Figure 1, that is proximal to distal on the X chromosome, and Pirrotta and Bröckl have located five exons between coordinates -2.1 and 3.6 . The positions of these exons are shown in Figure 1. O'Hare *et al.* (1984) have reached similar conclusions from analysis of the sequence of the *white* locus.

The two mutations which determine a bleached white phenotype, w^{IR7} and w^{IR8} , are associated with deletions affecting one or more exons and would not be expected to have any *white* gene function. The site of the 5.4-kb insertion associated with w^{IR4} is indistinguishable from that of the insertions associated with w^{IR1} and w^{IR3} (Bucheton *et al.*, 1984). We have shown by DNA sequence analysis that the I factor associated with w^{IR1} is inserted 96 bp to the right of the *Sal*I site at coordinate -1.4 (D. Fawcett and D.J. Finnegan, unpublished data) and so clearly lies within an intron. These three mutations determine an identical red-brown eye

colour, indicating that they do not abolish *white* gene function completely. They may interfere with RNA processing and reduce the level of full length *white* mRNA. Pirrotta and Bröckl (1984) and Levis *et al.* (1984) have shown that this is true of the w^a mutation which is due to insertion of a *copia* element within the short intron at coordinate 0. Alternatively, *white* mRNA might terminate within these insertions and code for a partially active *white* gene product.

We cannot say exactly where the insertions associated with w^{IR5} and w^{IR6} are in relation to *white* mRNA sequences. The w^{IR5} insertion is either just downstream of the mRNA coding sequence or just within the last exon, and may affect the termination of transcription or the C-terminal end of the *white* gene product. The w^{IR6} insertion is either at the 3' end of the first exon or the beginning of the first intron. It is the first mutation giving an altered eye colour to be mapped between the w^a and w^h mutations (O'Hare *et al.*, 1983). The fact that this mutation determines a coloured eye phenotype suggests that it is within the intron and reduces the level of wild-type *white* mRNA. Interruption of the *white* gene product near its N-terminal end would probably inactivate it completely. The exact position of these insertions will be determined by DNA sequencing and their effects on *white* mRNA measured directly.

The coloured phenotype of the w^{IR2} mutation is particularly interesting since it is a derivative of w^1 which gives a bleached white phenotype. The w^1 mutation is associated with insertion of a 5.7-kb transposable element 0.5 kb to the right of the *Hind*III site at coordinate 3.2 (Zachar and Bingham, 1982; Pirrotta and Bröckl, 1984). This is related to the 'F' family of elements (O'Hare *et al.*, 1983; Di Nocera *et al.*, 1983). In w^{IR2} there is a second insertion just within, or immediately to the right of, this F-like element. Two other derivatives of w^1 give coloured eyes. These are w^h and w^e (for references, see Lindsley and Grell, 1968). They are both associated with rearrangements within the F element. The w^h allele has a 1.1-kb deletion while the w^e insertion is 0.2 kb longer than that of w^1 and has a slightly altered restriction map.

The w^1 , w^e and w^h insertions are very close to the start of the *white* gene mRNA (O'Hare *et al.* 1983; Pirrotta and Bröckl, 1984; Levis *et al.*, 1984). Levis *et al.* (1984) and O'Hare *et al.* (1984) believe that these insertions lie just within

a short 5'-untranslated leader sequence. These mutations have been assigned to a regulatory region of the *white* locus since they suppress the phenotype of the *zeste* mutation, *z¹*. In addition, the *w^e* mutation does not show dosage compensation, unlike *w^h* and *w^{IR2}* (Lindsley and Grell, 1968; Smith and Lucchesi, 1969; Judd, 1976). The *w^e* allele directs synthesis of the major mRNA from the *white* region, as expected from its coloured phenotype, but the level of this RNA is greatly reduced (Pirrotta and Bročkl, 1984). We expect that the same will be true of *w^{IR2}*.

We have now analysed a total of eight *white* gene mutations induced in SF females. Six of these are associated with insertion of similar, if not identical, 5.4-kb sequences, and two are deletions. We have presented evidence previously which strongly suggests that the insertions present in *w^{IR1}* and *w^{IR3}* are genetically active I factors (Bucheton *et al.*, 1984). The only other mutation to have been tested for activity is *w^{IR2}* (mutation 1a11 in Pélişson, 1981), and it is not linked to an active I factor. This could be because the I factor present in *w^{IR2}* differs from functional I factors by one or more base substitutions or small deletions or insertions which would not have been detected in these experiments, or because its activity is prevented by adjacent DNA. The F-like element may inhibit its expression, for example.

The genomes of both inducer and reactive strains contain many I elements, at least some of which are incomplete compared with I factors. The results of whole genome Southern transfer experiments indicate that most I elements are located at very similar chromosomal sites in all strains suggesting that they transpose rarely, if at all (Bucheton *et al.*, 1984; Crozatier, Vaury and Bucheton, unpublished data). This could explain why we have not found any *white* gene mutations associated with I elements. These results differ from those obtained by Rubin *et al.* (1982) for P-M induced *white* mutations. They found that all six mutations they investigated were associated with insertions but that the elements involved were not identical. Four were different deletion derivatives of the P factor, and two were *copia* elements. This suggests firstly that P elements can transpose more readily than I elements and secondly, that P-M dysgenesis can mobilise *copia* elements and possibly other transposable sequences. There is no reason to suppose that this is true of I-R dysgenesis.

Mutations *w^{IR1}*, *w^{IR3}* and *w^{IR4}* are due to insertion of I factors at apparently identical sites at coordinate -1.3, suggesting that this is a hot-spot for insertion. This may be because I factor insertion is to some extent sequence specific. There is a hot-spot for P element insertion at coordinate -1.9, and P elements can insert at this site in either direction. The I factors at -1.3 are all in the same orientation but the sample is too small to say whether or not this is significant.

The deletions present in *w^{IR7}* and *w^{IR8}* are either the direct result of I-R dysgenesis or arose fortuitously in SF females. The majority of I-R induced *white* mutations are associated with a recessive lethal phenotype and are probably deletions (A. Pélişson, unpublished data). This suggests that I-R dysgenesis can induce deletions at this locus. Chromosome rearrangements induced by P-M dysgenesis often have P elements at their breakpoints (Bingham *et al.*, 1982) but we have been unable to find any I factor sequences associated with *w^{IR7}* and *w^{IR8}*. *In situ* hybridisation experiments have given no indication of I factor sequences associated with these mutations, although this does not rule out the presence of up to two or three hundred bases of I DNA. There is no detect-

able I factor DNA in the wild-type *white* locus at positions corresponding to the end points of these deletions. Perhaps they were formed by secondary events shortly after insertion of I factors at the *white* locus.

Materials and methods

Bacterial strains

All plasmids were propagated in *Escherichia coli* HB101 (Boyer and Roulland-Dussoix, 1969) and recombinant phages in strains C600 (Appleyard, 1954), Q359 (Karn *et al.*, 1980) and NM514 (Arber *et al.*, 1983).

Drosophila strains

All strains of *D. melanogaster* are from the collection of Laboratoire de Génétique, Université de Clermont-Ferrand.

Enzymes and isotopes

Restriction enzymes were purchased from Bethesda Research Laboratories, Amersham International and New England Biolabs and were used as recommended by the manufacturers. T4 DNA ligase was purchased from New England Biolabs. *E. coli* DNA polymerase was the gift of B.M. Will. [α -³²P]dCTP (410 Ci/mmol) and [³H]dCTP were purchased from Amersham International. [³H]dTTP was purchased from C.E.A. Sacay, France.

DNA preparation

Plasmid and phage DNAs were prepared as described by Will *et al.* (1981) and Maniatis *et al.* (1982). *D. melanogaster* DNA was prepared as described by Bucheton *et al.* (1984).

Agarose gel electrophoresis

Horizontal slab gels were run in Tris/acetate buffer (40 mM Tris, 20 mM Na acetate, 1 mM EDTA, pH 8.2) at ~1 V/cm. DNA was transferred to nitrocellulose by the modification of the method of Southern (1975) described by Smith and Summers (1980). Fragments of λ cl₈₅₇ DNA digested with *Hind*III were used as size markers.

In vitro labelling of DNA, hybridisation and autoradiography

These procedures were carried out as described by Will *et al.* (1981) except that dextran sulphate (mol. wt. 500 000) was present at a concentration of 3% during hybridisation. After hybridisation filters were washed for 2 h in 2 x SSC, 0.1% SDS at room temperature, and then for a further 2 h in 1 x SSC, 0.1% SDS at 37°C.

Construction of libraries

A library of cloned *Hind*III fragments of *w^{IR5}* DNA was constructed by ligating 1 μ g *Hind*III-cut λ NM1149 DNA with 1 μ g *Hind*III-cut *w^{IR5}* DNA, in a total volume of 15 μ l. Ligation was carried out overnight at 10°C in 100 mM Tris-HCl pH 7.2, 10 mM EDTA, 100 mM MgCl₂, 100 mM dithiothreitol, 10 mM ATP and with 60 units T4 DNA ligase. Recombinant molecules were packed *in vitro* (Scherer *et al.*, 1981) and the resulting phages were plated on *E. coli* strain NM514. The library was first screened with pCS54 to recover phages containing fragments from the appropriate region of the *white* locus. Phages hybridising to this probe were then tested with pCS157 and pI769 to detect those containing sequences from the right or left of the *w^{IR5}* insertion, respectively.

A library of cloned *Bam*HI fragments of *w^{IR2}* DNA was made as follows. 10 μ g of EMBL4 DNA was digested with both *Bam*HI and *Sal*I and the resulting polylinker fragment was eliminated by precipitating once with ethanol (Frischauf *et al.*, 1983). This vector DNA was dissolved, together with 3 μ g *Bam*HI-cut *w^{IR2}* DNA, in 100 μ l ligation buffer and ligated overnight at 10°C. The ligated DNA was packaged *in vitro* and the resulting phages plated on *E. coli* strains Q359 and C600. Plaques were screened with ³²P-labelled pI768. Phages hybridising to this probe should contain the *Bam*HI fragments immediately to the left or right of coordinate 1.5. Phages carrying the left-hand fragment were identified by screening with pCS156. One of the phages, λ I531, which did not hybridise to the probe was taken for further analysis.

In situ hybridisation

Hybridisation to polytene chromosomes was carried out as described by Pardue and Gall (1975) as modified by Bucheton *et al.* (1984).

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